14 March 2011
EMA/CVMP/ERA/81952/2011
Committee for Medicinal Products for Veterinary Use (CVMP)

Overview of comments received on Guideline on determining the fate of veterinary medicinal products in manure (EMA/CVMP/ERA/430327/2009-Rev.1)

Interested parties (organisations or individuals) that commented on the draft document as released for consultation.

Stakeholder no.	Name of organisation or individual
1	Vetpharma Animal Health, S.L.
2	RIVM
3	IFAH-Europe
4	Wildlife

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1. General comments – overview

Stakeholder no.	General comment (if any)	Outcome (if applicable)
1	Information is missing regarding the procedure to calculate the concentration of the active in manure. The equation in the CVMP guideline (p23) gives only a mass and not a concentration. This value needs to be divided by the amount of manure produced by the animal in a specific time period. This is the information that is missing from the degradation in manure guideline. We will probably obtain different expected concentrations in manure depending on the animal category within the same species. Therefore, which value should we take to perform the study: the mean, minimum or maximum?	The section is revised to give more guidance on how the test results can be used to calculate PEC soil. We again recognize that this is not explicitly mentioned in the guidance. Ideally, the DT50 is concentration independent and in that case the highest dose could be used unless this is insufficient to measure the transformation products. It might be possible that around the maximum predicted concentration the microbial activity is affected. In such case it will be necessary to test lower doses as well when for certain target species lower PECs are expected The fact that the maximum concentration should be tested has been included in the guidance
3	A more aligned approach towards fate testing of VMPs in manure is welcomed, even though, as acknowledged in the draft guideline (section 1.2), insufficient experience is present at this point in time, and many knowledge gaps still exist. Therefore, not only should the guidance be considered a living document, but it is equally important to maintain flexibility in the study design and interpretation, and not require strict adherence to the present document for a study to be valid. Any study should be judged on its own scientific quality and merits to encourage scientific progress and innovation.	We agree. We also would like to emphasize that this is a guidance document, providing general recommendations on how to perform a degradation study in manure and should therefore not be considered as a technical guideline as such. Some technical details included in the draft of the guidance document have been removed and might be included in the future in a technical guideline. We fully recognise that our current knowledge on the factors affected degradation in manure is still limited and therefore adjustments might be necessary in the future.

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	The requirements within the guideline should be acceptable throughout the EU region. There is a risk of setting guidance criteria which may not ensure that studies are acceptable in all areas/countries – i.e. the requirement for anaerobic conditions for cattle manure may not be acceptable in areas where cattle are kept in feedlot situations, which also is the case in certain parts of the EU. There is a risk of diverging criteria creating a need for multiple degradation studies to be conducted.	The requirement for anaerobic conditions is considered representative (Weinfurtner 2010, see section 2.3.2) and realistic worst case and will thus be acceptable throughout the EU.
	Further clarification may be necessary on the requirements and test conditions for some of the target animal species. For example: 1) Sheep are mentioned a few times in this guideline, but the suggested dry matter content was not given for sheep manure. Furthermore, they are not considered to be housed animals under the CVMP guidance (EMEA/CVMP/ERA/418282/2005-Rev.1). 2) Since the horse is a minor species, can the results obtained in e.g. cattle be extrapolated to horses?	Points 1 and 2. This guidance document attempts to standardise the degradation tests for manure from housed animals. Therefore, sheep and horse are not considered.
	3) In case an Applicant wishes to perform manure degradation studies for some of the minor target species (horses, rabbits, etc.), are there any suggestions for their test conditions?	3. In the absence of sufficient information, as yet, no guidance is given for rabbit manure.
	4) Guidance on poultry manure is largely missing (e.g. bedding?)	4. We agree that the information on the characteristics of chicken manure is missing. For practical reasons, we gave recommendation on the test conditions considering that these reflect the average condition in a stable. If more information or other considerations need to be made the ERAWP/CVMP are open for any additional information and suggestions from stakeholders on this point

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	As a general rule, DT50 (and/or DT90) end points should be reported along with mineralization rate, 14C-mass balances, and major degradate formations/identifications where appropriate. This guidance document requires far more information to be collected in the degradation studies than can be used in the risk assessment. The study protocol should be simplified to collect only the information that is useful. For example, identification of degradation peaks and repeated extractions using various solvent mixes and validated procedures provides no useful risk assessment information and is very expensive. Unless an Applicant is able to synthesize the degradation product(s) in sufficient quantities for ecotoxicology testing, which would be very expensive and perhaps not even possible, the degradation products over 10 percent are treated like the active ingredient for risk assessment purposes anyhow.	We included an additional section (§3.1) to clarify how data should be treated Concerning metabolites we agree that identification of degradation products is not necessary based on the general approach that worst case they could be considered as being parent compound. This is now included in the revised version of the guideline.
4	Overall, the draft guideline is well written and comprehensive. It has been our experience that the analytical methods (including extraction and clean up) are the most challenging aspect of degradation in manure studies. Changes in the test system (manure) over relatively short periods complicate analytical methods and do not allow for the type of pre-test validation normally associated with degradation studies performed in soil or sediment water systems. It is often necessary to modify extraction procedures or add clean-up steps during the test based on observed changes in extractable components of the matrix (e.g. soluble organic matter). Our main concern with the guideline as written is with the wording pertaining the validation of analytical methods and identification of transformation products. The importance of having validated extraction and analytical methods is acknowledged. However the guideline should be reasonably flexible given the difficult nature of the test system.	

2. Specific comments on text

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
Section 1.1	2	Comment: In this section it should be defined what the goal of the determination should be. Depending on the goal of the study, different approaches may be important. In our view, the goal of the study can not be to determine bound residues in manure, and to use these for refinement of risk assessment. When referring to bound residues, please use the definitions and terms as agreed upon during the recent ECETOC workshop on bound residues: http://www.ecetoc.org/index.php?mact=MCSoap,cntnt01,details,0&cntnt01by_category=6&cntnt01template=display_list_v2&cntnt01order_by=Reference%20Desc&cntnt01display_template=display_details_v2&cntnt01document_id=2379&cntnt01returnid=93 Definitions according to ECETOC workshop (2009): Extractable residue: A residue that is extractable using 'mild' extraction methods. This may include aqueous and cold solvent extraction using methods without excessive added energy. These residues are either freely available, or only weakly adsorbed to the matrix, are considered to be bioavailable and must be considered in any impact / risk assessment. Non-extractable residue: A residue that is not extractable using 'mild' extraction methods, but	We would like to emphasize that this is a guidance document and not a technical guideline. It is up to the applicant to determine the goal of the study. We agree that the applicant should make this clear in the introduction of the study report. As the VICH phase II provides the possibility to add the metabolites to the parent compound, it might not be necessary to identify the metabolites as long as this total residue approach is followed. We do however fully recognise the importance of the extraction techniques and the difficulties of using the non-extractable fraction (including the use of non-radiolabelled material) in the refinement of the risk assessment. We have tried to reflect this throughout the document following a conservative approach. Based on the comments given we do however consider to extend this issue to provide as much guidance as possible on the design and interpretation of the results to avoid miscommunication between the study submitter and the risk assessor. As stated, the definitions proposed during the ECETOC workshop might not work for a manure matrix and by using them as such they might confuse readers.

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		extractable under harsher conditions. These conditions may include solvent extraction using methods such as refluxing, microwaves or accelerated solvent extraction (ASE). These residues are strongly associated with the matrix, however they may be potentially reversible; but the partitioning is very much in favour of 'binding' to components of the matrix. Therefore, for risk assessment purposes, this matrix associated fraction is unlikely to be available to indigenous organisms. Bound residue: A residue that is tightly associated with the solid matrix, often forming covalent (or similar) bonds. These residues usually cannot be released from the matrix or can only be released under extreme conditions where the integrity of the substance and/or matrix is likely to be affected. Such residues are often indistinguishable from the natural organic material e.g. humus in soil. These residues are not available for either degradation or available for indigenous organisms and should not be considered in any impact / risk assessment. Thus, according to these definitions, only bound residue can be used to refine risk for soils. But: please note that according to the participants of the workshop, in manure both bound and non-extractable residue do not exist at all; because of the nature of the manure all parent compound should in time be released. Employing these definitions means that the use of radiolabelled analytes is inevitable.	

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		If the goal of the study is to determine (a)biotic degradation rate constants and/or the identification of metabolites, this should be stated explicitly. For instance, when the goal is to identify metabolites and to use these (in quantities lower than 10%) to refine risk, this can only be done when radio-labelled material is used and a complete mass balance can be presented.	
Section 1.1	2	End of 1st subsection: "if complete degradation or mineralization can be demonstrated". These are two very different objectives combined in one sentence. A substance can be completely degraded but not be mineralised. If complete degradation of the substance has taken place, there might be fractions of metabolites > 10% present. These are to be taken into account in the risk assessment, following the adopted principle of the total residue approach. Please alter the wording to 'complete mineralisation' or 'complete degradation with all degradation products < 10%' or alike.	The text has been changed to "…This type of study is not required but it could be used to stop the assessment in Phase I in case it can be demonstrated that the active is mineralised or degrades into products present < 5%, If the data does not allow to stop the assessment in phase I it could still be useful to refine the exposure assessment in Phase II. …"
Section 1.2	2	Comments: 2nd subsection. Other striking differences that should be mentioned are: (i) the way manure is being collected and stored and (ii) the variation in composition due to the type of feed the animals have been given.	Agreed, these points have been added to the guidance.
Section 1.2	3	While some general guidance on the conduct of manure degradation studies and their use in risk assessment is welcome, insufficient research exists to absolutely narrow down requirements. This would discourage	This guidance document gives only general information and no specific details on testing. General requirements of the guideline should be followed.

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		companies from investing in manure degradation studies and research on this compartment of environmental relevance. Therefore, consider adding wording to the final paragraph to permit deviation from this guideline, such as “consequently, the Applicant may deviate from this guidance in order to improve the study design, providing that adequate scientific justification is presented”.	
Section 2.1	2	Comments: 2nd subsection, last line. "The feed type, feeding regime and the veterinary history of the animals from which the manure is collected should be recorded." Please add: and reported	Agreed, the guidance now indicates “… should be reported”
Section 2.1	2	3rd subsection. " it is recommended that manure is reconditioned at ambient conditions for 3 days and the matrix characterised again". Please clarify: characterised again after the 3 days at ambient conditions?	With characterisation is meant to measure the parameters listed in section 2.2. This reference will be included.
Section 2.1	3	Should there be any comment on whether or not litter or straw should be included or removed from the sample (more important for chickens)? Should test systems be faeces, faeces+urine, or faeces+urine+bedding? Can metabolism with one type of bedding for poultry be extrapolated to other test systems with different bedding? Slurry versus dung pats? Why does the manure need to be “conditioned” and	Good questions, though difficult to answer. Little is known about the effect of the variety of constituents in manure on the degradation of VMPs. At the moment, there is the need for a standardised transformation tests in liquid manure that can be advanced during the next years by gathering more detailed experiences. Not any real situation can be simulated at the same time. Here, this may be a misunderstanding. In the draft of the

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		what is the definition of “conditioned”? If a conditioning step is necessary, then this precludes study designs which measure the depletion of a VMP in manure from treated animals, i.e., degradation of the VMP may take place during the “conditioning” process. There are strong indications that microbial content/activity of the manure is a factor of major significance in the degradation process for a number of substances (as is the case for soils). “Conditioning” treatments such as freezing of the manure can influence/alter this microbial content/activity and impact the degradation rate. This is also not done with soils. It is not clear why manure should be acclimated for 21 days; as soon as stable anaerobic conditions are reached (as measured by the redox potential), the study could be started. There is also a contradiction regarding reconditioning after freezing; a study can only start when stable anaerobic conditions are reached and that may require more time. It would be very helpful to provide definitions of “test manure” and “reference manure” as this terminology is	guideline, the technical terms "manure", "reference manure" and "test manure" are used without any clear differentiation to avoid any predefinition of two different approaches: i. Reference Manure Concept Here, excrements were taken from test animals. Those excrements are matrix characterised and conditioned for 21 days to establish strictly anaerobic conditions and to reduce the matrix heterogeneity by transformation of readily degradable organic substances. Subsequently, tap water is added to prepare reference manure samples of defined dry substance contents. Of course, here the technical term "standard manure" or "test manure" could be defined by the regulatory authorities. However, those technical terms should not be used alternating to avoid confusion. ii. Tank manure concept Samples are taken from manure tanks. There, neither "conditioning" nor "acclimatisation" are necessary when sampling occurred in tanks under strictly anaerobic conditions. " ...freezing of the manure..." This may be another misunderstanding due to indistinct wording. In the Reference Manure Concept, only excrement samples were stored at -20 °C up to one year, then reconditioned, prepared to reference manures and successfully tested for matrix characterisation and VMP or biocide transformation. Nevertheless, fresh excrement or manure samples may be preferred for regulatory studies. The text has been adjusted to provide more clarity. .

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		currently not clear.	
Section 2.1 Page 4/9	4	Comment: The last sentence states that tap water should be used. In many places residual chlorine is present in tap water. Proposed change (if any): Suggest changing to laboratory water.	Agree, text has been adjusted (deionised water).
Section 2.2	2	"it is important that the following parameters are measured." Please add: and reported. Please add: Temperature Moisture content When should these parameters be measured? Propose: at start and end of test, not only at the beginning. Some parameters may be monitored throughout the test (e.g. temperature).	Agree with the proposed wording. Temperature will be added. As moisture content is related to dry matter content (% dry matter content + % moisture content = 100 %), moisture content is redundant. Agree with the proposal. Other parameters to be monitored throughout the test are pH and redox potential (such as in OECD 307 and 308)
Section 2.2	3	It has already been acknowledged in the draft guideline that the effects of the waste characteristics are generally unknown, so collecting characteristics such as nitrogen content seems unlikely to be useful in evaluating the representational adequacy of tests done with different compounds. Microbial activity could be estimated by biomass, or could be estimated by gassing-off of CO_2 or CH_4, or	Several parameters of matrix characterisation have to be analysed in accordance to analytical quality assurance. Nitrogen may one of them besides ds, pH, Eh, O_2, TOC, microbial activity. The footnote are just examples on how to determine the microbial activity.

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		plate counts (although with plate counts, there is a risk of overestimating real activity) could be included. In analogy with what is done for soils when conducting a study in line with OECD 307, a standard such as radiolabelled glucose or sodium benzoate could be used to evaluate microbial activity and determine the appropriateness of the manure sample (and the viability of the test system as the study progresses). This has already been done successfully. Therefore, the statement in footnote 2: "… causes other problems" needs to be explained or a reference cited. What are the other problems?	The problem is: The application of a readily degradable reference substance, e.g., sodium benzoate, in parallel batch experiments causes other inadequacies. In order to check the microbial activity of manure samples at the start of the transformation test series, this test is too time consuming due to its 4-week test period specified by the OECD Guideline 311. Due to the different experimental designs, – the degradability of sodium benzoate is only measured by the gas production –, this test is not appropriate to check the microbial activity at longer incubation intervals
		The microbial activity should be tested to reflect the actual test conditions used for manure degradation, for example, aerobic versus anaerobic. One way is to test the microbial activity parallel to the experiment conditions and to ensure the manure and test condition used are suitable to degrade known reference compounds.	Those "reference compounds" should be ^{14}C-labelled and should show a characteristic transformation within the 100-day test period because at test termination, it is to be checked if manure samples are microbially active.
		The guideline allows the use of manure from animals already receiving the test product to be considered. In this way the microbial population in the test manure will be representative of the situation when the product is in use.	Using manure from treated animals is not recommended because this complicates the analytical determinations and in many cases no meaningful results will be obtained.
		It might be useful to measure phosphate content as well, as it might have some correlation with microbial activity. If redox potential is measured, oxygen measurement may not be necessary. Guidance on	This also applies to nitrogen content. We consider the nitrogen content to be sufficient. Redox potentials of manure are in the range from -230 to -400

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		acceptable redox potential may be useful to avoid discussion on whether “acceptable” anaerobic conditions have been reached or not. Is the purpose to set a range for acceptable “microbial activity”, and, in the affirmative, what would that be?	mV (Weinfurtner 2010). Thus, test redox potentials should be below -200 mV. The guidance document will be amended to avoid case-by-case discussion on that issue. This is intended to realise reproducible test conditions. The general guidance document does not set a range for acceptable microbial activity. Further data sharing is welcome and information might be included in a latter technical guidance.
Section 2.2 Page 5/9	4	Comment: It is often useful and in some instances more appropriate to determine test system characteristics at different stages throughout the study. For example, determining microbial activity or biomass at the end of the study allows for an assessment of any changes in the viability of the test system over the test period. Proposed change (if any): Present parameters to be measured and stages of test that the measurements should be performed as done in OECD test guideline 308. Comment: It is not clear if dissolved oxygen of slurry or oxygen content within the headspace of the test vessels should be measured. Proposed change (if any):	We have added to following text: All the parameters should be measured at the start and termination of the study. In accordance to OECD 307 and 308, it is recommended to monitor pH, redox potential end temperature throughout the test. Dissolved oxygen content should be measured

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Section 2.3.1	3	As discussed in previous meetings, spiking the chicken may be a valuable option for poultry.	This will make the experimental design more difficult. How many test animals has to be introduced to compensate the biological variability. Assuming that an administered VMP is transformed and parent compound and 3 metabolites are excreted as relevant substances (>10 %), the regulatory authorities have to make the decision if the test substance mixture or every single substance should be tested for transformation in manure.
		Sacrifice of individual test vessels at each time point is procedurally easier to do than sub-sampling a single vessel. However, parameters of 50 g minimum fresh weight and 5% solids content would result in large vessels. This is manageable, but it results in a large volume to extract and potentially use of a large amount of radiolabelled material.	50-g manure batch experiments can be performed in vessel, e.g., 300-ml Erlenmeyer flasks. This is the same for testing transformation in soil. In any case, sub-sampling should be avoided in order to guarantee reproducible analysis.
		The guidance should include an example of the typical number of replicates and sampling intervals.	Agreed. We propose to follow the recommendations of OECD 307. In the OECD guideline, two flasks per time points are recommended. It is mentioned that the time intervals should be chosen in such a way that pattern of decline of the test substance and patterns of formation and decline of transformation products can be established (e.g. 0, 1, 3, 7 days; 2, 3 weeks; 1, 2, 3 months, etc.). In addition, if results of the tests have to be specified for a certain day for PEC refinement (e. g. half storage period) then it is recommended to take a sample on that day.
Section 2.3.2	2	Discontinuous is not understood.	Discontinuous has been replaced by flow-through system

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Section 2.3.2	3	There is concern about the acceptance of the aerobic/anaerobic split within and outside of the EU. Manure from cattle and pigs should not be specifically required to be tested under anaerobic conditions, perhaps representing just waste collection slurry pits. Requirements to make cattle and pig manure anaerobic using nitrogen might not be representative of more aerobic situations, such as manure deposited in animal pens, cattle kept under feedlot conditions etc.	As mentioned above , the current guidance is focussed on manure form housed animals At least 50% of the manure in the EU are (semi) liquid manure (mixture of faces, urine and water) and is to be considered to be the most relevant type of manure. In central European countries this type of manure might represent even higher percentages. Therefore (semi)liquid manure is considered to be representative (Weinfurtner 2010). This has been added in the general considerations (§ 1.2).
Section 2.3.2 Page 5/9	4	Comment: Measurement of redox potential can be problematic in some cases and may have neither a mechanistic nor a predictive value and noted in OECD TG 308. Proposed change (if any): Add option for measurement of $^{14}CH_4$ production from a reference substance as evidence of anaerobic conditions.	In a footnote of §2.2 it is added that the microbial activity can be determined by measuring the mineralisation of a readily degradable ^{14}C-labelled compound (e.g. ^{14}C-glucose) under anaerobic conditions
Section 2.3.3	2	"Poultry manure will normally contain a much higher dry matter content" should read Poultry manure will normally *have* a much higher dry matter content.	Agree with the proposed wording, changed accordingly.
Section 2.3.3	2	Footnote 2 "Microbial activity could be proven" Please choose different wording. Measured, quantified, demonstrated, expressed, etc.	See previous comment
Section 2.3.3	3	Dry matter contents – the value for pigs is only representative of slurry conditions. The dry matter	We assumed that pig slurry is most representative for investigation degradation in a storage tank. Weinfurtner 2010 has gathered information on dry matter content and

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		content of faeces alone is closer to 35%. For poultry the range of dry matter is in the order of 30- 60%. A justification for the choice of the dry matter numbers is missing; this can not be based on one (peer-reviewed?) publication alone. If slurry conditions are to be used for manure degradation will the application of the results to dung pats at pasture be acceptable? Suggested values should be given for manure from sheep and other species.	concentrations of 5 % and 10 % are considered a realistic assumption for pigs and cattle. Concerning poultry manure the proposal was considered to be a reasonable average dry matter content. We recognise that one peer review is limited and could be extended with more data. We are open for more information and willing to reconsider the proposed dry matter content if needed. As mentioned above , the current guidance is focussed on manure form housed animals
Section 2.3.4	3	We agree the test duration should be driven by the rate of degradation of the compound under study, and should aim at the determination of a DT50, regardless of average manure storage times mentioned in the TGD (which are very artificial, not based on real practice, potentially subject to change and not applicable everywhere). However, as a guidance in absence of any pilot data with the compound under study, 120 days could be considered in analogy with soils, provided that the test system remains sufficiently viable –an issue well known from degradation studies in soil.	We do not accept that average manure storage times in the TGD are “very artificial”. We agree that without any information upfront a duration of 120 days could be considered in analogue with OECD 307. Propose to add this in the guidance.
Section 2.3.5	3	Standardisation to a test temperature of 20°C could be acceptable but may require further discussion (see also section 3.2.5)	Noted
Section 2.3.6	2	This is most relevant when the study is conducted with non-radiolabelled test substance. Please use different wording. Inclusion of sterile	It is agreed that sterile controls are beneficial for the interpretation of results from degradation studies in manure. It is also noted that consideration should be given to the

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		controls is evenly relevant when a radiolabelled analyte is used. Sterile controls should simply be part of the test set up.	sterilisation method used in order not to change matrix properties too much. Furthermore, manure sample may not remain sterile when the study is conducted over a long period.
Section 2.3.6	3	Including a sterile control should be optional. Whether degradation is biotic or abiotic is of little consequence for risk assessment purposes – the VMP either degrades or not. What methods of sterilization are recommended? It would be appropriate that any point of guidance which is seen as critical be included in the present guideline. The reference to Kreuzig et al at the end of this section can only be an illustration, as this is not a validated test method. Reference to OECD guideline 307 could also be made.	In some cases sterile controls are necessary for interpretation of results from the degradation studies in manure. This is why we continue to recommend their inclusion. However, it is only a recommendation so it is up to the applicant whether to include them or not. We have added that consideration should be given to the sterilisation method used in order not to change matrix properties too much. Furthermore, one should be aware that manure samples may not remain sterile when the study is conducted over a long period. For sterilisation methods reference is made to OECD 307. Of course, the reference to Kreuzig et al. is only one illustration. Further experiences to be gained in regulatory testing are necessary. All critical parameters and test conditions are included in the guidance. Again we would like to emphasize this document is not a technical guideline and deviations are possible when justified.
Section 2.4	2	1st subsection, first line: "because detailed mass balances can be determined". A full mass balance should be determined in all studies and this is most easily (for most compounds probably only) achieved when using a radiolabelled analyte. " For the determination of 14C-methane released out of the 14C-labelled test substance, the 14C-carbon dioxide	Noted. Text changed to “Radiolabelled material is preferred because detailed mass balances can be determined taking into account mineralisation, extractable and non-extractable residues”.

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		free headspace of the incubation flask has to be transferred" This is not understood (technically) by us, how can methane be trapped from a 14CO2 free headspace? In general both CH4 and CO2 will be formed, and are thus both present in the headspace. If CO2 has been blown out by the continuous N2 stream, any CH4 will have also disappeared. A schematic drawing of the test set up would be helpful for the guideline in general.	Agreed, the technical recommendation is further clarified.
Section 2.4	2	2nd subsection "or, when necessary, in minimum amounts of an organic solvent" Please indicate what the minimum amount is to avoid discussions when evaluating the studies.	In our view it goes beyond this guidance document to list all organic solvent and there toxicity to bacteria. In fact the same recommendation is given in OECD 307. By measuring the microbial activity it could be determined whether the solvent significantly inhibits the microbial activity. Like in OECD 307 we could give advice against the use of chloroform, dichloromethane and other halogenated solvents, which inhibit microbial activity, such as, should be avoided. In order to provide some recommendation we need some additional information on the toxicity of solvents on microbial activity.
		If a solvent is used, a solvent control should be included in the test set up. Please report this. However note that under anaerobic conditions some organic solvents can be used as carbon source, which might lead to cometabolism or other disturbance of the desired degradation pattern.	It will be added that the use of a positive control could be of help to determine the toxicity of the solvent.
		If non-radiolabelled material is used, please state here that metabolites then may be identified but data cannot be used for quantitative risk refinement.	The current text already states: *"When non-radiolabelled material is used, only the disappearance of the parent compound initially applied can be followed unless specific*

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			methods exist for known transformation products". This is considered sufficient.
		Characterise the formation of bound residues: by definition (see comments on section 1.1), this is not possible in manure. If bound or non-extractable residues are measured, probably not the right extraction technique has been used. The choice of extraction technique therefore is very important and should be discussed in the study report. For definitions of bound residues and NER, see ECETOC workshop report.	Agreed, the section on extraction methods will be expanded reflecting this. We have highlight in § 3.3.4 that, in contrast to soil and sediment, manure will be degraded to a large extent after application to soil. Therefore, matrix attacking harsh extractions might be helpful to assess long-term behaviour.
Section 2.4	3	We suggest clear instructions are given on the test apparatus recommended, for example, flow-through systems with humidified air for aerobic manure degradations, stop-flow modes for anaerobic manure degradations with determination of ^{14}C-methane formation, etc.	Such technical details go beyond a more general description of the study design which hampers the flexibility needed. Such detailed descriptions will be included in any technical guideline which may be prepared. Such detailed descriptions will be included in a latter technical guideline.
		The characteristics of the radiolabelled material should be stated (number of labels versus number of possible label sites; is the molecule uniformly labelled, ring labelled or just labelled on a carboxylic or methyl group?) since the results of the study must be interpreted based on the placement and extent of labelling. It should also be noted that in the case of fermentation products, radiolabelling is not a simple exercise, and the position of the labelling can not always be specified.	We have added that phenylring-U-14C labelling should be preferred. If such is not feasible, side-chain labelling – ^{14}C-methyl labelling is preferred to ^{14}C-carboxylic labelling – is the second choice. 3H-labelling may be a further alternative. In all cases the characteristics of the radiolabelled material should be reported.
		Often, ADME studies and/or soil degradation studies will	

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		have been conducted; the labelling used in these studies should be acceptable for manure degradation studies as well.	
Section 2.4 Page 6/9	4	Adsorbent materials are commonly used to trap ^{14}C volatile substance. In addition, the combustion apparatus described for anaerobic conditions may be used for an aerobic test to trap volatile organics. Proposed change (if any): Revise wording accordingly.	Text has been adjusted accordingly.
Section 2.5	2	"The primary objective of the study is" The primary objectives of the study are, etc. Please adapt, since all mentioned goals are valuable objectives. E.g. it does not only concern degradation of the active. E.g. if it is demonstrated that the parent is degraded, but no CO2 is measured, the total residue approach can not be abandoned and the PEC can not be reduced. This is not a hypothetical case.	Agree with the proposed wording.
Section 2.5	2	"An exhaustive extraction is necessary with various apolar and polar solvents and acid systems." Strongly suggest to add text like: the choice of which depends on the analyte. First extraction steps can employ less rigid methods (e.g.) short time shaking with (e.g. organic) solvens, however more rigid methods should be employed in order to destroy the manure matrix in case bound residues are observed. The more rigid methods employ e.g. pressurized liquid extraction, reflux, soxhlet, etc. with	Agreed, guideline revised in accordance.

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		the appropriate solvents. Suggest to add last step. The residue remaining after the last extraction step should be combusted and analysed for evolving CO2. The possible fraction of radiolabel contained therein is termed non-extractable residue (NER).	
Section 2.5	3	The sentence “An exhaustive extraction is necessary” should be clarified to reflect that these are extraction options and all the possible extractions listed need not be performed. Extraction will remain a difficult issue for a number of substances, certainly after a certain time of incubation. Therefore, more specific guidance is needed. If possible, examples should be given which extraction steps should be used and after which level of harshness extraction attempts can be stopped. If products have been selected for identification as they are present at > 10%, they must already have been “quantified”. Suggest “quantified” is deleted. The current text ignores the analytical difficulties encountered in a very complex matrix, where both quantification and identification may not be straightforward. An alternative way of dealing with this issue could be the following: A lower limit could be given for quantification. Attempting quantification down to 0.01 mg/kg seems reasonable. This will limit the requirements for the specific activity of the radiolabelled test substance to be	Improvements have been included in the guidance. It is recognised that it is not always necessary to identify the metabolites when a worst case approach is followed by considering them as parent compound. The text in this section has been revised. The revisions to the text hopefully address all the points made here.

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		used. Next, there must be a lower limit for characterisation and identification. The following is proposed: • If the concentration is lower than 0.01 mg/kg, there is no need to make efforts for characterisation or identification. • If the concentration exceeds 10%, attempts must be made to extract the residue and to characterise the extractable residue fractions, this means obtain data on partition behaviour and chromatographic information to allow a prediction of similarity with the parent substance. • If the concentration is higher than 0.1 mg/kg or 10 % of the applied dose, whichever is greater, attempts must be made to characterise and identify the residue fraction (in analogy with metabolism studies). Many times it is not possible to verify the identity of transformation products, or the cost and time to do this could be prohibitive. Since in those cases, for the risk assessment these metabolites are added to parent concentrations, identification failure should not be seen as invalidating the whole study and its further use in the risk assessment. Identification is more important in cases where only a major metabolite remains, than for metabolites just over 10% of administered dose, and this should be seen in its proper perspective. The following statement is unclear: “Analytical methods for identification and quantification of the test	

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		substance and its transformation products should be available." Does this mean that they should be included in the risk assessment, that they should be done prior to the manure degradation study (not knowing which transformation products might occur during the study in the first place), or that they should be available from other functional areas? Ideally validated methods for transformation products are available, but that is not always possible given that they might occur at such low levels that it is impossible to isolate, purify them and then do method validation. This requirement exceeds those for e.g. ADME studies, unless it refers to studies conducted with non-labelled material?	
Section 2.5 Page 7/9	4	Comment: It is often not possible to identify all transformation products. The relatively small amounts of transformation products recovered and matrix background from these types of studies can preclude extensive analytical investigation. In addition, analytical standards are not always available for microbial degradation products. Proposed change (if any): Suggest adding wording that the transformation products should be identified unless reasonably justified otherwise.	Text has been adjusted to meet this comment
Section 2.5 Page 7/9		Comment: The intent of the statement "The validation of the analytical methods should be the same for both the parent compound and the transformation products" is not clear. Analytical methods (including extraction	Sentence has been deleted.

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		and clean-up) for the parent material are typically developed with radiolabelled material. Rarely are radiolabelled transformation products available. Proposed change (if any): Clarify statement.	
Section 3.1	3	How is demonstration that "the composition of manure and storage conditions are comparable" seen in practice? This sentence currently does not provide any guidance and by default will give rise to a variety of interpretations for that requirement.	We agree, but the following sentences have been included to give further recommendation: "At present there is limited information available to provide more precise guidance for which species and manure types extrapolation is feasible. For pragmatic reasons, manure within the same animal type, i.e. pigs, cattle, sheep and poultry is considered to be comparable". Although from a scientific point of view this could be criticized.
Section 3.2	2	Second bullet. Why are complete mineralisation and complete degradation taken into one line? This suggests that these are comparable results, while they are actually very different.	The current text is fully in line with the recommendations of the CVMP guideline. No changes are made …
Section 3.2	2	Fourth bullet. Is this not equal to 'partially degraded'?	No, primarily degradation of the parent compound could mean that the parent compound is completely transformed into degradation products.
Section 3.2	2	Please do not mix up the terms (non-)extractable and bound residues, but use the definitions from the ECETOC workshop.	As already mentioned we do not think it is appropriate to use the definitions from the ECETOC workshop for manure studies.
Section 3.2.2	2	In our view, this is a little too cryptic. – At what point in time should the percentage $_{CO2}$ formed be taken? Suggest to take half the storage time from Table 6 of CVMP technical guidance as a measuring point. Extrapolation is not accepted, the	We recognise that this needs some further clarification. For phase I the formation of CO_2 after the default storage time of one month should be taken. For phase II the storage times mentioned in table 6 of the CVMP guideline should be taken. It is thus important to measure at half maximal storage time for

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		study should last long enough to cover ½*Tstorage for each of the target animals within a target animal group. – Should the mean of replicate CO_2 percentages at a given point in time be taken or the worst-case amount? Or should the fitted amount of CO_2 be used. – How should the PEC be recalculated?	Phase II. We propose to use the mean of replicate CO_2 percentages at a given point - see response on comments of Vetpharma
Section 3.2.2	3	Any percentage mineralisation will be subjective, and will depend on the position of the labels. Given that it is by far unrealistic to assume that every compound can be labelled on every carbon atom, mineralisation should be viewed in its proper perspective. When just a (few) side chain(s) of a complex molecule are labelled, that is a completely different situation than when a molecule is uniformly ring-labelled. In the former case, the side chains may be split from the molecule, but the core structure of that molecule may be still intact. Seeing a % of mineralisation in the case of ring-labelling has a far greater meaning and indicates that the molecule is capable of being ultimately degraded to H2O and CO2. This is information that is qualitatively extremely relevant to the risk assessment, but just a general refinement based on % mineralisation is not a valid scientific approach. Quantitative PEC-refinement should be based on degradation, not on mineralisation.	See labelling discussion above
Section 3.2.3	2	Third line "this rule can also be applied". Please change into: This rule also applies to	Text has been adjusted, making this comment no longer relevant.

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Section 3.2.3	2	Fourth line. "However, when NER remain.." Here it is very important what NER means. It should thus be defined. Otherwise there will be continued discussion on the refinement of PEC values between regulators and IND. If NER is defined as above, this would mean that any residual radioactivity designated "NER" can never be subtracted from the total radioactivity and is always a part of the PEC. However, when actually bound residues are meant (as described above), these may be subtracted from total radioactivity. However, if the set-up of a study (i.e. extraction techniques employed) is such that the fraction does not meet the definition, the radioactivity contained in the residue after extraction can not be subtracted from the PEC as it might still contain degradation products that might be released in the field (upon degradation of manure). And again, please note that refinement with the bound residue fraction is only deemed valid for soils.	Noted. Text will be amended to make this more explicitly clear.
Section 3.2.3	2	2nd subsection. Major transformation products. When is a transformation product 'major'? Is this also the 10% level? If two or three (etc.) major metabolites are formed in manure (these may also have been formed in the animal), 'ecotoxicity' is not the only concern. The metabolites (generally of increasing polarity) may reach ground- and surfacewater more easily.	This section has been adjusted to provide more clarity on the approaches that can be followed. In line with the VICH it is not compulsory to identify the metabolites as long as the total residue approach is followed.

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		" these compounds have a similar activity to the parent compound unless it can be shown that the ecotoxicity of these compounds is less than the parent compound" Of course, if something has been demonstrated, we no longer have to assume it (for the specific case). I.e. we can use toxicity data for the metabolite if these are present. Or is something else implied here? It would probably be best not to give guidance on requirements for metabolite testing in the ERA in this guideline. We suggest to be more clear here or to remove the sentence.	
Section 3.2.3	3	How is PEC refinement seen in practice here? Refinement options should be clear. The (desired) outcome of a degradation study is a DT50; this result is subsequently used in an exponential equation to refine the PECsoil. In analogy with combination products, PECsoil could be refined for parent and each of the relevant major metabolites using equations 9 to 11 of the TGD, and then summing up the individual soil PECs derived from this. Mi for metabolites could be the highest concentration observed in the study, refined to Mt using their respective half-lives. In those instances where transformation products cannot be synthesized for further fate (radiolabelling??) and effects studies, testing with the parent should remain an acceptable alternative. Likewise, if complete degradation to a metabolite takes place, it should be optional for the Applicant to use the metabolite for risk	The text has been adjusted to provide more clarity on the use of the test results, considering the comments given.

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		assessment purposes. In most cases, significant testing with parent drug will have taken place before results from such a manure degradation study will be available. It is logical to assume (with the exception of pro-drugs, which are covered elsewhere) that the parent compound will present the greatest toxicity. Suggest to delete the sentence: "However, when NER remain, this rule should be applied with a certain restraint considering that these residues might still contain transformation products which when added to the extractable part will comprise a percentage > 10%". As it is very difficult to characterize and quantify the degradation products in the NER, in practice it is not feasible to determine a percent value for a particular degradation product in the NER and then add it to the extractable portion. Thus, this requirement will be very difficult to achieve. Also, the NER should not be considered as immediately bioavailable and added as additional parent compound. The NER may be released when the manure matrix degrades, but it is at a later time when most of the other compounds are degraded in soils.	
Section 3.2.4	2	"manure will be degraded to a large extent" after application to soil. "might not be appropriate for manure" is not appropriate for manure.	Agree with the proposed wording.

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Section 3.2.4	2	Second section Beware of definitions again. NER is the fraction that may become available (e.g. upon degradation of manure in soil). Bound residue will not become available. Writing about "NER that will not cause any toxicity when manure degrades.." is thus confusing. This should read "Bound residues, that will not cause any toxicity when manure…". Please rewrite subsequently (e.g.) The fraction that may still become available after spreading manure onto land (NER), should be considered to represent the parent compound unless it can be shown, through appropriate experiments, that the toxicity of the NER is significantly lower than the parent compound.	Noted.
Section 3.2.4	3	Considering non-extractable residues as being equivalent to parent seems to be very conservative. It seems likely that as the compound is broken down, the parts are used as building blocks for microbial metabolism. Sorption behaviour could be taken into account here as well. If a compound is very mobile in soil, the likelihood that the NER will largely consist of fragments incorporated eg in bacteria is high, whereas for compounds that adsorb to particles, adsorption may indeed be a contributing factor in the disappearance of substance in a manure degradation test. Also if no NER are formed over time in the sterile controls, then that might be a strong indication towards potential incorporation of fragments into organic matter.	It all depends what is observed in the degradation study to argue on what could represent the NER. If no degradation is observed, NER could still contain parent compound. When degradation is observed it could be parent, metabolites or incorporation into microbials. The higher the percentage of CO2 formation the higher the likelihood that indeed fragments are incorporated. It also depends on the severity of the extraction methods used to provide assurance that the maximum amount of parent compounds and metabolites have been extracted. We have adjusted the text taking the comments into account

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		Showing that non-extractable residues are less ecotoxic than parent is almost impossible. It is stated that: "Initiatives are under development": will there be any scientific exchange/discussion with stakeholders? Should this not form an integral part of the present guideline, as it is an issue that will be encountered in virtually every degradation study in manure? It should also be borne in mind that it is generally accepted (chemicals, plant protection products, pesticides, RIVM document 601516007) that extraction efficiency should be at least 70%, and NER up to 30% are considered to be falling within the method variability. The same rationale should apply here in this case.	We know that attempts are made to test the toxicity of VMPs to terrestrial species with and without (stored) manure to investigate whether the non-extractable fraction is still toxic / bioavailable. As yet, more discussion is needed to provide more guidance. For this reason the sentence has been deleted. Acceptance of an extraction method is something else than disregarding a percentage of NER. This reasoning would also encourage using less appropriate extraction methods. Furthermore, 70% extractability is really the lower limit.
Section 3.2.5	3	Some more explanation on the equation and the validity of suggested assumptions from the EFSA PPR Panel to this particular situation would be appropriate, thereby noting that the PPR Panel has concluded that there are group-specific and compound-specific differences in Ea, and the opinion proposes values specifically for plant production products. In analogy with soil degradation testing (OECD 307), a standard test temperature of 20 ± 2 °C would be acceptable. For the purpose of refining PEC calculations for the manure storage, IFAH-Europe does not consider it necessary to change the temperature from 20 ˚C to	We agree that ideally the substance specific activation energy should be used, but often this is not available. Considering that a large variety of pesticides have been analysed we believe it is appropriate to use the default value as proposed by the PPR panel in the absence of other data. As the use of the equation and a default value is already mentioned in the CVMP guideline we believe that further guidance is not necessary, but we will mention in the footnote that more information can be found in the PPR opinion. Based on the findings of Weinfurtner, 2010 we believe that 10 ˚C is a realistic storage temperature for pig manure and therefore remain to our position that the conversion from 20 ˚C to 10 ˚C is needed.

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		10 ˚C for pigs in the CVMP guideline EMEA/CVMP/ERA/418282/2005-Rev.1). However, for the sake of standardization and avoiding the conversions of DT50 values for various temperatures, we agree it would worth revising the CVMP guideline also using 20 ˚C for all species so that the data obtained from the tests can be directly used for calculations. This temperature could also be seen as an average temperature overall; in a pile or a tank, a range of temperature gradients will be present. Therefore, and given the fact that the Ea to be used is not necessarily applicable to veterinary medicines, the absolute need to correct DT50 with the Arrhenius equation merits at least some further discussion.	
Section 5	2	There should be a more official guidance document for analytical validation techniques than the factsheet by De Knecht et al. Consider replacing.	Reference is given to the guidance for generating and reporting methods of analysis of the EC (2000).
Section 5	3	The last two references should be separated out, one for VICH GL38 and the other for CVMP guideline. “GL38 (EMEA/CVMP/ERA/418282/2005-Rev.1)” should be added at the end instead of “GL3”.	Corrected.